Heart Rate Variability and Spontaneous Baroreflex Sequences: Implications for Autonomic Monitoring During Hemorrhage

William H. Cooke, PhD, and Victor A. Convertino, PhD

Background: Noninvasive procedures for predicting progression to hemodynamic instability during induced central hypovolemia in humans were evaluated. The purpose of this study was twofold: (1) to track changes in autonomic function induced by a model of hemorrhage, and (2) to determine whether measures of autonomic function are reliable without strict control of breathing.

Methods: Electrocardiogram, respiratory frequency, and arterial pressure during progressive lower body negative pressure were recorded for 10 subjects, and for a separate sample of 20 subjects during 5-minute periods of spontaneous breathing or controlled-frequency breathing at 15 breaths per minute.. Heart rate variability was calculated in both time and frequency domains. Up and down baroreflex sequences were calculated with linear regression analysis between sequential changes in systolic pressures and accompanying parallel changes in R-R intervals.

Results: Heart rate variability ($r^2 = 0.92$) and up ($r^2 = 0.90$) and down ($r^2 = 0.96$) sequences changed in direct inverse relation to decreased central volume as produced by progressive increases in lower body negative pressure, whereas mean arterial pressures remained constant ($r^2 = 0.26$). Neither heart rate variability nor up and down baroreflex sequences were affected by the mode of breathing.

Conclusions: Analysis of heart rate variability and baroreflex sequences in hemorrhaging patients may provide advance recognition of those at risk for progression to shock. In conjunction with more traditional modes of assessing volume status, tracking early changes in autonomic function may improve resuscitation efforts for the hemodynamic compromised patient.

Key Words: Arterial pressure oscillations, Autonomic, Power spectral analysis, Blood pressure control, Lower body negative pressure, Hemorrhagic shock.

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Maintenance of hemodynamic stability during traumatic hemorrhage depends on functioning autonomic reflexes. Thus, tracking changes in autonomic function may detect patients progressing to hemorrhagic shock. For example, heart rate variability analysis shows promise as a technique for probing autonomic mechanisms during trauma, as several recent studies have demonstrated associations between heart rate variability and outcome predictions.[1–3] Winchell and Hoyt[4] estimated relative vagal and sympathetic contributions to heart rate variability using frequency domain analysis with a large cohort of patients admitted to an intensive care unit (~740). Survival was associated with low vagal and high sympathetic tone, and death was associated with high vagal and low sympathetic tone.[4] Likewise, responsiveness of the cardiac baroreflex has been shown to reflect autonomic drive, and analysis of cardiac baroreflex sensitivity may be useful as a diagnostic tool for hemorrhagic patients. Cardiac baroreflex sensitivity (BRS) may be assessed noninvasively from linear regression analysis of sequences of three or more progressively increasing or decreasing systolic pressures (measured with finger photoplethysmography) and consequent increases or decreases in R-R intervals.[5]

The magnitude of change in BRS, as measured using noninvasive "baroreflex sequences," may prove useful as a tool to predict the onset of hemorrhagic shock, but at least two methodologic issues must be overcome. First, obvious ethical constraints prevent the controlled study of autonomic function during progressive bleeding to cardiovascular collapse in humans. To overcome this limitation, we propose the use of lower body negative pressure (LBNP) as a method for replicating the hemodynamic and autonomic effects of hemorrhage. Application of LBNP sequesters blood in dependent regions of the pelvis and legs, and induces magnitudes of central hypovolemia and autonomic compensations similar to those of acute hemorrhage.[6]

Second, accurate quantification of autonomic rhythms depends importantly on respiratory frequency, so subjects for such experiments are required to control their breathing frequency at a constant rate.[7,8] A recent study demonstrated that spontaneous breathing, as compared with voluntary apnea,

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increased the number of baroreflex sequences,[5] suggesting that breathing frequency may influence the validity of baroreflex sequence analysis. The influence of controlled versus uncontrolled breathing on the occurrence of baroreflex sequences has not been determined.

In an attempt to determine whether tracking autonomic function can predict the progression to hemorrhagic shock, this study examined noninvasive assessments of autonomic responses to progressive central hypovolemia by LBNP and the influence of controlled frequency breathing on autonomic responses. The results suggest that linear reductions in ongoing vagal activity, assessed with either heart rate variability or baroreflex sequences, may represent a readily identifiable early marker that tracks progression to hemorrhagic instability in bleeding patients who maintain unchanged arterial pressures.

MATERIALS AND METHODS
Subjects

To assess the influence of progressive central hypovolemia on autonomic function (experiment 1), this study analyzed data from 10 men between the ages of 27 and 52 years (mean, 39 years) during exposure to progressive LBNP. To assess the influence of controlled breathing on autonomic function (experiment 2), the study analyzed data from 20 different men and women between the ages of 18 and 26 years (mean, 22 years) during spontaneous breathing (subjects were allowed to set their own cadence) and during controlled-frequency breathing (subjects breathed at a rate of 15 breaths per minute). All the subjects read and signed consent forms approved by the human research committees of Michigan Technological University or the U.S. Army Institute of Surgical Research. All the subjects were nonsmokers with no history of autonomic dysfunction. The subjects reported to the laboratory after they had abstained from caffeine, exercise, and alcohol for at least 12 hours. All measurements were made with the subjects in the supine position.

LBNP as a Hemorrhage Model

We have proposed the use of LBNP as an experimental tool for simulating hemorrhage in humans.[6] Absolute equivalence between the magnitude of negative pressure applied and the magnitude of actual blood loss cannot currently be determined, but review of both human and animal data shows ranges of effective blood loss (or fluid displacement) caused by LBNP. On the basis of the magnitude of central hypovolemia induced, we previously proposed that 10 to 20 mm Hg of negative pressure is equivalent to blood loss ranging from 400 to 550 mL, that 20 to 40 mm Hg negative pressure is equivalent to blood loss ranging from 550 to 1,000 mL, and that negative pressure exceeding 40 mm Hg is equivalent to blood loss approximating 1,000 mL or more.[6] We also attempted previously to classify hemorrhage as mild, moderate, or severe, and to coordinate these descriptors with the magnitude of chamber decompression during LBNP. A thorough review of such comparisons can be found in our previous work,[6] but for convenience, the primary results from their review are produced in Table 1.

Experimental Protocol and Analysis

All the subjects were instrumented with an electrocardiogram (ECG) and a finger photoplethysmograph (Finapres, Model 2300, Ohmeda, Englewood, CO) to record beat-by-beat arterial pressure. The subjects in experiment 2 also were instrumented with an abdominal bellows to record respiratory rate (uncalibrated strain gauge pressure transducer).

Table 1 Comparison of Global Physiologic Responses to Hemorrhage and Lower Body Negative Pressure (LBNP)[a]

Classification Hemorrhage LBNP Variable	Stage 1 Mild (10 to 20 mm Hg)	Stage 2 Moderate (20 to 40 mm Hg)	Stage 2 Severe (>40 mm Hg)	Stage 3 Shock (Collapse)
HR	**↑** ↔	**↑** ↑	**↑** ↑	**↑** ↓ ↓
MAP	**↔** ↔	**↑** ↔	**↑** ↑	**↓** ↓
SV	**↓** ↓	**↓** ↓	**↓** ↓	**↓** ↓
Q$_c$	**↓** ↓	**↓** ↓	**↓** ↓	**↓** ↓
CVP	**↓** ↓	**↓** ↓	**↓** ↓	**↓** ↔ ↓ ↔
SNA	**↑** ↑	**↑** ↑	**↑** ↑	**↓** ↓
NE	**↑** ↑	**↑** ↑	**↑** ↑ ↔[b]	**↓** ↓
PVR	**↑** ↑	**↑** ↑	**↑** ↑	**↓** ↓
AVP	**↔** ↔	**↔** ↔	**↔** ↔ ↑[b]	**NA** ↑
PR	**↔** ↔	**↔** ↔	**↔** ↔ ↑[b]	**NA** ↑
ANGII	**NA** ↔	**NA** ↔	**NA** ↑	**NA** ↑
PPH	**↔** ↔	**↔** ↔	**↑** ↑	**↑** ↑

NA, data not available or too limited to present; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; Q$_c$, cardiac output; CVP, central venous pressure; SNA, sympathetic nerve activity; NE, norepinephrine; PVR, peripheral vascular resistance; AVP, arginine vasopressin; PR, plasma renin; ANG II, angiotensin II; PPH, pancreatic polypeptide hormone.

[a] Under each condition, variables either increase (↑), decrease (↓), do not change (↔), or show differential responses (↓ ↔; ↔ ↑). Responses to hemorrhage are shown in **bold** font and responses to LBNP are shown in normal font.

[b] Directional changes only in subjects susceptible to hemodynamic collapse or at the onset of hypotension.

(Table reproduced from Cooke WH, Ryan KL, et al. 2003 628/id).

For experiment 1, the subjects underwent an LBNP protocol consisting of a 12-minute baseline period followed by exposure to 15, 30, 45, and 60 mm Hg of decompression for 12 minutes each, followed by return to baseline (0 mm Hg) for 12 minutes. For 3 minutes during each stage, the subjects controlled their breathing rate at a strict 15 breaths per minute (0.25 Hz) for assessment of heart rate variability.[7] Breathing at 15 breaths per minute may have been faster than the subjects' normal unpaced breathing rate, but the study purpose was to ensure that oscillations of R-R intervals occurring at the respiratory frequency were not confounded inappropriately by harmonics of low-frequency rhythms occurring at approximately 0.1 Hz. During this experiment, the first 2 minutes of each stage were used for experimental retinal scans (data not presented), and the subjects responded verbally to instructions from the investigators. For this reason, there are no data for a comparison of heart rate variability between uncontrolled spontaneous breathing and controlled-frequency breathing during LBNP.

For experiment 2, the subjects were supine for a 5-minute stabilization period. Data then were recorded with the subjects breathing spontaneously at an uncontrolled rate for 5 minutes. After this, the subjects breathed in time to a metronome set at a pace of 15 breaths per minute (0.25 Hz) for an additional 5 minutes. These data were used to assess the influence of controlled-frequency breathing on heart rate variability and spontaneous baroreflex sequences and BRS.

Data were sampled at 500 Hz, recorded directly to a computer with commercial hardware and software (WINDAQ, Dataq instruments, Akron, OH), and then imported into data analysis software (WinCPRS, Absolute Aliens, Turku, Finland). Next, R waves generated from the ECG signal were detected and marked at their occurrence in time. Systolic pressures subsequently were marked from the Finapres tracing.

Heart rate variability was assessed in the frequency domain from R-R interval spectral power. The R-R intervals were made equidistant by spline interpolation and resampling at 5 Hz. Data then were passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. To obtain power spectrums, 3-minute data sets (experiment 1) and 5-minute data sets (experiment 2) were fast Fourier transformed with a Hanning window. Heart rate variability was quantified as the total integrated area within the high-frequency (0.15–0.4 Hz) band.[9,10]

Automated computer analysis was used to search the entire data record for potential baroreflex sequences. A potential valid sequence was defined as three or more progressively increasing or decreasing systolic pressures with at least a 1-mm Hg change per beat and associated R-R intervals with at least a 4-ms change per beat.[5] Sequences of increasing systolic pressures and R-R intervals were classified as "up sequences," whereas decreasing systolic pressures and R-R intervals were classified as "down sequences." Cardiac baroreflex sensitivity (gain) was estimated with linear regression analysis. Only sequences with correlations of 0.8 or greater were considered to be valid sequences and included in the analysis.[5]

Statistical Analysis

All data were analyzed with commercial statistical software (SAS Institute, Cary, NC). For experiment 1, regression coefficients were calculated between LBNP and R-R-interval high-frequency power, spontaneous baroreflex sequences, and mean arterial pressure. In addition, analysis of variance (ANOVA) was used for repeated measures to compare heart rate variability at baseline with heart rate variability during the highest LBNP level (-60 mm Hg). For experiment 2, for differences between the means of each dependent variable were tested using a two-way ANOVA with repeated measures of both condition (uncontrolled breathing vs. controlled breathing) and time (1-minute periods). Significance was set at a p value of 0.05 or less. Data are presented as means $\pm$ standard error of the mean unless specified otherwise.

RESULTS
Experiment 1

Figure 1 shows the original tracings of arterial pressures and ECG. Systolic pressures and R-R intervals are shown in the upper two panels, with valid up sequences marked in the R-R interval panel.

Figure 2 shows R-R intervals and associated frequency domain representations for one representative subject during baseline (0 mm Hg), -60 mm Hg decompression, and return to 0 mm Hg. The data shown in Figure 2 represent the magnitude of reduction in R-R interval spectral power at -60, as compared with 0-mm Hg chamber decompression. As a group (n = 10), the average magnitude of reduction in heart rate variability from 0 mm Hg to -60 mm Hg was similar to the example shown in Figure 2 and statistically significant ($p = 0.0001$). For all the subjects, LBNP caused progressive decreases in both heart rate variability and BRS for both up and down baroreflex sequences, as shown in Figure 3. Heart rate variability and BRS were correlated inversely with LBNP level ($r^2 = 0.92$ for LBNP and heart rate variability; $r^2 = 0.90$ for LBNP and baroreflex up sequences; $r^2 = 0.96$ for LBNP and baroreflex down sequences). Mean arterial pressure did not change predictably with progressive LBNP ($r^2 = 0.26$ for LBNP and mean arterial pressure).

Experiment 2

Breathing frequency was not significantly different between uncontrolled and controlled frequency breathing. The average respiratory rate was 15.5 $\pm$ 0.9 breaths per minute during spontaneous breathing and exactly 15 $\pm$ 0.0 breaths per minute (by design) during controlled breathing ($p = 0.8$). Controlled breathing did not affect estimates of vagal cardiac control. The R-R intervals were 953 $\pm$ 26 ms during uncontrolled breathing and 942 $\pm$ 29 ms during controlled breath-
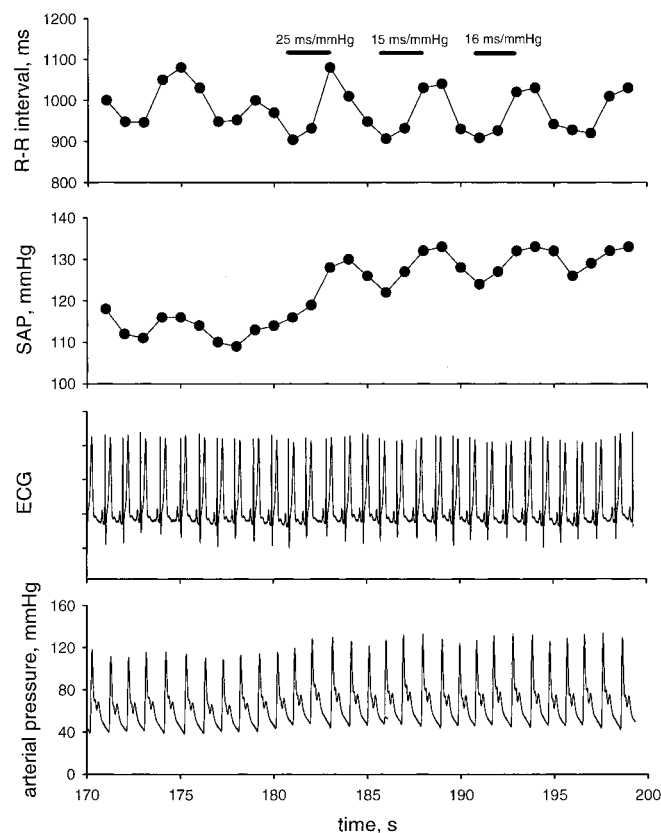
Fig. 1. *Representative tracings of the electrocardiogram (ECG) and finger photoplethysmpgraphic arterial pressures are shown in the bottom two panels. Derived systolic pressures (SAP) and cardiac interbeat intervals (R-R interval) are shown in the top two panels. The horizontal line in the R-R interval panel shows R-R intervals associated with valid (r ≥ 0.8) up sequences calculated from linear regression analysis between three progressively increasing systolic pressures and R-R intervals.*

ing ($p = 0.2$). The findings showed R-R interval standard deviations of 69 ± 8 ms during uncontrolled breathing and 65 ± 7 ms during controlled breathing ($p = 0.1$), and an R-R interval high-frequency power of 1837 ± 573 ms^2 during uncontrolled breathing and 1410 ± 339 ms^2 during controlled breathing ($p = 0.3$).

Controlled breathing did not affect the number of up or down baroreflex sequences or BRS. An average of 13 potential up sequences and 11 potential down sequences were detected during the 5-minute periods of both uncontrolled and controlled breathing. The percentage of these sequences determined to be valid up sequences (those with $r \geq 0.8$) were not affected by controlled breathing ($6.4\% \pm 0.8\%$ uncontrolled vs. $7.8\% \pm 1.3\%$ controlled; $p = 0.4$), nor were the percentages of valid down sequences thus affected ($6.8\% \pm 0.9\%$ uncontrolled vs. $6.6\% \pm 0.8\%$ controlled; $p = 0.9$). Cardiac baroreflex sensitivity calculated for up sequences (29 ± 4.1 ms/mm Hg uncontrolled vs. 21 ± 2.1 ms/mm Hg controlled; $p = 0.9$) and down sequences (21 ± 2.2 ms/mm Hg uncontrolled vs. 17 ms/mm Hg controlled; $p = 0.1$) were statistically indistinguishable between the two conditions.

No condition by time interaction effects were found. Figure 4 shows characteristics of baroreflex gains across time, and Tables 2 and 3 show the number of up and down sequences identified, the percentage of valid sequences, and the mean BRS for each minute during each condition.

DISCUSSION

Monitoring of pulse rate, mentation, and arterial pressure may be inadequate for the assessment of injury severity in bleeding patients. Although altered mentation and low blood pressure are used currently to assess injury severity, "normal" blood pressure has long been recognized as being a poor indicator of blood loss.[11,12] A need exists for an algorithm or procedure to predict the onset of hypotension and impending hemodynamic instability during hemorrhage. Changes in au-
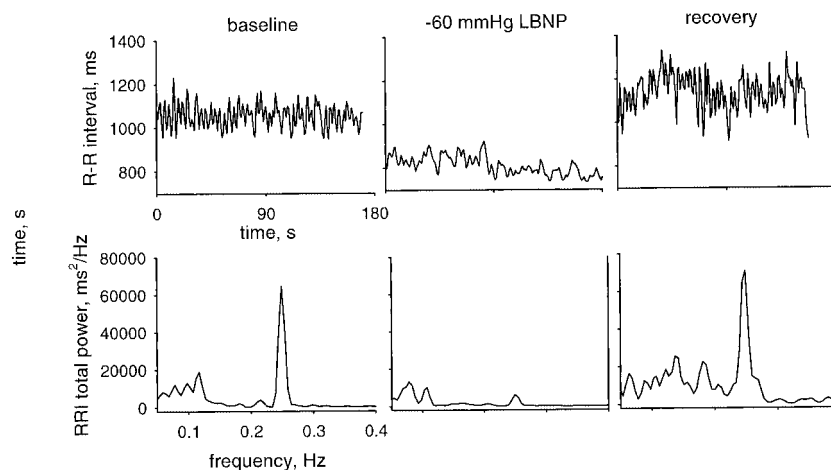
Fig. 2. *For one subject, 3-minute examples of R-R intervals are plotted during lower body negative pressure (LBNP). Associated frequency domain representations of R-R intervals (RRI total power) are shown below each time series.*
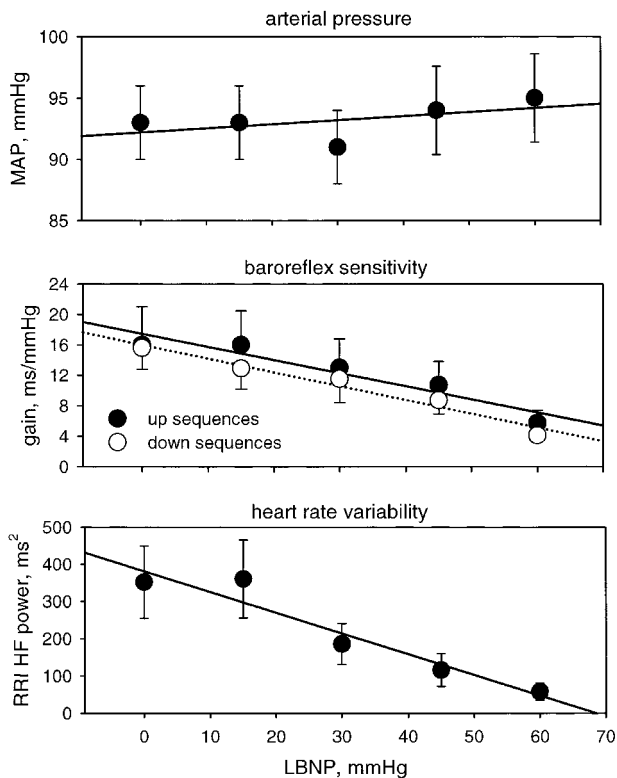
Fig. 3. *The effects of progressive central hypovolemia induced by lower body negative pressure (LBNP) are shown for mean arterial pressures (MAP), cardiac baroreflex sensitivity of up and down sequences, and integrated R-R interval spectral power at the high frequency (RRI HF power, 0.15–0.4 Hz) (n = 10).*

tonomic function may provide important information. We conducted studies of human autonomic function to understand better how heart rate variability and BRS change as functions of progressive central hypovolemia. They also studied the influence of controlled frequency breathing, an experimental technique used to assess human autonomic rhythms to determine whether interpretation of these autonomic measurements depends on maintenance of a steady breathing rate. The results demonstrate that heart rate variability and BRS change are direct inverse functions of LBNP magnitude, whereas mean arterial pressures remain constant. In addition, heart rate variability and BRS are not affected importantly by controlled breathing.

We conclude that heart rate variability and baroreflex sequence analyses accurately represent autonomic changes occurring during progressive central hypovolemia, and may have greater predictive power than measures of arterial pressure for early identification of progression to hemodynamic instability. Accurate application of heart rate variability and baroreflex analyses does not depend on maintenance of an unchanging breathing rate, and therefore show promise as tools for assisting in the assessment of hemodynamic status in bleeding patients.
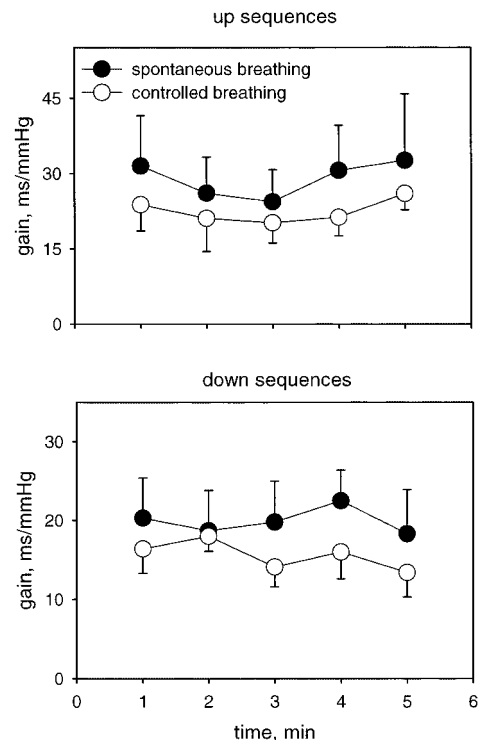
Fig. 4. *The effects of controlled-frequency breathing (0.25 Hz) versus uncontrolled spontaneous breathing are shown for up and down cardiac baroreflexes over the course of 5 minutes (n = 20).*

Autonomic Responses to Central Hypovolemia Induced Through Application of LBNP

The greatest utility of using LBNP as a model to replicate hemodynamic effects of hemorrhage is shown by comparing compensatory responses between the two conditions. Both hemorrhage and LBNP induce central hypovolemia in proportion to blood loss or negative pressure applied, and the resulting compensations include sympathoexcitation to increase peripheral vascular resistance and heart rate to counteract reductions of stroke volume and defend arterial pressure (Table 1). Autonomic sympathetic activation is fundamental to the maintenance of hemodynamic stability under both conditions, and both hemorrhagic shock during actual hemorrhage and hemodynamic instability with high-level LBNP occur consequent to abrupt hypotension mediated by sympathetic neural withdrawal.[13–15]

Figure 3 shows that both heart rate variability and BRS change early, and in direct inverse proportion to the magnitude of LBNP (i.e., central hypovolemia). Mean arterial pressure is effectively maintained at a constant level, and is therefore of little use for an early prediction algorithm. The results, shown in Figure 3, suggest that changes in autonomic vagal activity could assist in the early assessment of hemorrhage severity.

Heart rate reflects average ongoing sympathetic neural traffic with a time delay of about 10 seconds attributable to intrinsic delays in effector responses to norepinephrine.[16] In

Table 2 Characteristics of Up Sequences During Uncontrolled, Spontaneous, and Controlled-Frequency Breathing

	Time (min)	Sequences[a] n (range)	Valid[b] (%)	Mean[c] ms/mm Hg (n)
UB	1	3 (0–7)	5.9 ± 1.9	31.5 ± 10.0 (11)
	2	2 (0–5)	5.7 ± 1.7	26.2 ± 7.2 (11)
	3	2 (0–5)	7.3 ± 1.9	24.5 ± 6.4 (11)
	4	3 (0–4)	7.1 ± 2.0	30.6 ± 9.0 (13)
	5	3 (0–5)	6.3 ± 1.9	32.6 ± 13.2 (11)
CB	1	2 (0–5)	8.1 ± 1.6	23.8 ± 5.2 (15)
	2	3 (0–4)	5.4 ± 1.5	21.8 ± 6.6 (11)
	3	3 (0–4)	7.2 ± 2.4	20.2 ± 4.0 (10)
	4	3 (0–5)	8.2 ± 3.0	21.3 ± 3.7 (10)
	5	2 (0–3)	10.3 ± 4.5	25.4 ± 3.2 (11)

UB, uncontrolled, spontaneous breathing; CB, controlled-frequency breathing at 0.25 Hz.

[a] Number of valid sequences identified per subject with ranges shown in parentheses.

[b] Percentage ± standard error of all potential sequences (n = 20 subjects per time period) that met the criterion for a valid up sequence.

[c] Arithmetic average ± standard error of all valid up sequences with the number of observations contributing to that mean (n) shown in parentheses.

Table 3 Characteristics of Down Sequences During Uncontrolled, Spontaneous, and Controlled-Frequency Breathing

Condition	Time (min)	Sequences[a] n (range)	Valid[b] (%)	Mean[c] ms/mm Hg (n)
UB	1	2 (0–4)	8.4 ± 2.1	20.3 ± 5.1 (14)
	2	2 (0–3)	6.7 ± 2.1	18.7 ± 5.1 (10)
	3	2 (0–3)	7.0 ± 2.4	19.8 ± 5.2 (10)
	4	3 (0–4)	6.8 ± 9.6	22.5 ± 3.9 (11)
	5	2 (0–3)	5.4 ± 1.8	18.3 ± 5.6 (9)
CB	1	2 (0–4)	7.1 ± 1.6	16.4 ± 3.1 (15)
	2	2 (0–4)	6.6 ± 1.7	18.5 ± 1.9 (11)
	3	3 (0–4)	7.1 ± 2.3	14.2 ± 2.5 (11)
	4	2 (0–5)	6.2 ± 1.8	16.2 ± 3.4 (10)
	5	2 (0–3)	6.2 ± 1.4	13.4 ± 3.1 (12)

UB, uncontrolled, spontaneous breathing; CB, controlled-frequency breathing at 0.25 Hz.

[a] Number of valid sequences identified per subject with ranges shown in parentheses.

[b] Percentage ± standard error of all potential sequences (n = 20 subjects per time period) that met the criterion for a valid down sequence.

[c] Arithmetic average ± standard error of all valid down sequences with the number of observations contributing to that mean (n) shown in parentheses.

contrast, acetylcholine kinetics (including quick degradation by acetylcholinesterase) allow for autonomic vagal activity to modulate cardiac rate on a beat-by-beat basis in response to prevailing hemodynamic changes.[17] Heart rate variability, expressed in Figure 3 as the integrated area under the high-frequency R-R interval power spectrum, reflects primarily vagal cardiac activity.[18] Because of quick acetylcholine activation and inhibition in response to changes in arterial

distention, up baroreflex sequences reflect vagal activation, and down baroreflex sequences reflect vagal inhibition.[17] Both up and down baroreflex sequences decrease predictably as functions of LBNP magnitude (Fig. 3), suggesting that BRS conceivably could track progression to hemodynamic instability in bleeding patients.

The finding that loss of central blood volume is associated with an acute attenuation of BRS is not without precedent. In a previous study, exposure to 50 mm Hg of LBNP caused a 30% reduction in BRS that was reversed when central blood volume was restored with the use of G-suits inflated to 50 mm Hg.[19] Similarly, BRS measured during rest and exercise has been reduced with the application of LBNP (reduced central blood volume) and increased by the application of lower body positive pressure (increased central blood volume).[20,21] In contrast to the current investigation, BRS was measured in these previous studies[19–21] by applying pulse-synchronous neck pressure stimuli that allowed assessment of the isolated carotid cardiac baroreflex response over most of the reflex operational range. The findings of the current study extend the findings of previous experiments by demonstrating that spontaneous baroreflex sequences reflecting an integrated response of numerous baroreflexes may provide a simple, noninvasive early marker of acute alterations in central blood volume.

The attenuation of BRS may provide an important early marker for progression to hemodynamic instability. In the presence of an average 15% reduction of blood volume in subjects confined to bed rest, the largest reductions in cardiovascular insufficiency (hypotension and vasovagal syncope) were correlated with the greatest magnitude of reduction in vagal baroreflex gain.[22] In a similar fashion, low BRS represented one of the primary factors contributing to the prediction of early cardiovascular collapse.[21] Thus, the current findings of a linear relation between reduced BRS and central hypovolemia may be the first to suggest that spontaneous baroreflex sequences represent an early and continuous predictor of progression to hemodynamic instability.

Although the potential of using changes in spontaneous baroreflex sequences as a marker of blood loss is attractive, we recognize the limitation that their central hypovolemia model of LBNP fails to include the loss of blood volume associated with hemorrhage. With this model, there is no hole in a vessel. Of concern is the observation that an average reduction of approximately 500 mL in blood volume had no effect on carotid cardiac BRS,[23] suggesting that BRS may be influenced by fluid redistribution within the vascular space rather than by actual volume reduction. An attenuated BRS during LBNP is consistent with evidence supporting the existence of a muscle chemoreflex that would act to decrease systemic arterial pressure when circulation to the legs is improved.[19] Decreased heart rate response to baroreceptor stimulation (i.e., attenuated cardiac baroreflex gain) could represent one mechanism by which the muscle chemoreflex reduces arterial pressure. Because LBNP was used as a model

to simulate hemorrhage, the possibility that reduction in BRS with graded hypovolemia observed in the current investigation represents a specific response to fluid redistribution to the lower extremities cannot be dismissed. However, in a recent study, blood donation of 350 to 400 mL decreased BRS, as assessed with techniques identical to those outlined for the current study.[24] Blood donation reduced the sensitivity of baroreflex up sequences and tended to decrease the sensitivity of baroreflex down sequences.[24] Animal studies have documented reductions in baroreflex sensitivity, with blood loss exceeding 30% of total blood volume.[25–27] Thus, evidence from animal hemorrhage models coupled with the recent observations in humans[24] provides evidence that the reduction in BRS observed in the LBNP model may indeed represent a phenomenon of central hypovolemia rather than a chemoreflex response.

Influence of Controlled Breathing

Breathing frequency and depth may influence autonomic rhythms profoundly, so investigators have suggested that for research purposes, breathing rate should be strictly controlled.[7] Trauma patients clearly do not breathe at a set cadence, but the utility of heart rate variability analysis in real-life scenarios has nevertheless been shown.[1–3] At least one study has suggested that control of breathing decreases vagal cardiac control,[28] but at least two other studies contradict this notion.[8,29] It must be stressed that dramatic changes in R-R interval spectral power occur when breathing is slowed excessively and maintained at low frequencies.[7] Breathing frequency was not different during spontaneous and controlled breathing in the current study, but frequencies ranged from 10 to 20 breaths per minute when the subjects were allowed to breathe spontaneously. That R-R interval spectral power, R-R intervals, and R-R interval standard deviations were indistinguishable statistically between the two conditions suggests that such noninvasive measures of vagal cardiac control are appropriate even when breathing frequencies vary widely about a mean.

Rothlisberger et al.[5] recently showed that voluntary apnea significantly reduced the occurrence of spontaneous baroreflex sequences, as compared with spontaneous breathing. Before spontaneous baroreflex sequences can be recommended as a noninvasive diagnostic technique, comparable results between spontaneous breathing (real-life scenarios) and strict controlled-frequency breathing (laboratory scenarios) must be demonstrated. The current data show that breathing at a fixed rate has no effect on the occurrence of baroreflex sequences, on the percentage of valid sequences, or on the sensitivity of the baroreflex response. This is true for both up and down sequences (Tables 1 and 2, Fig. 4).

CONCLUSIONS

Heart rate variability was assessed with standard Fourier analysis, and baroreflex sensitivity was assessed with sequence analysis primarily because these techniques have been appropriately vetted in the literature and we have extensive experience analyzing and interpreting results of such analyses.[5,8,30–32] The results support the use of heart rate variability and baroreflex sequence analysis as potential markers of hemorrhage severity based on a hemorrhage model incorporating LBNP. However, the current study did not address the possibility that other techniques, such as approximate entropy or wavelet analyses for heart rate variability or cross-spectral transfer function magnitude for baroreflex sensitivity, may prove to be as useful or even more useful in a clinical setting. Future studies applying a broad range of measures for autonomic function could yield additional insight or improve the practical utility of the concepts outlined in this report. Additionally, although control of breathing frequency is recommended strongly for the accurate analysis of autonomic rhythms in the laboratory, such breathing control is not realistic in trauma care. The results show that heart rate variability and baroreflex sequence analyses are not confounded when responses during spontaneous breathing (with frequencies ranging from 10 to 20 breaths per minute) are compared with responses during controlled breathing at a set rate of 15 breaths per minute. We conclude that analysis of heart rate variability and baroreflex sequences during hemorrhage could serve as an important adjunct to the monitoring of pulse and blood pressure, and may develop into a reliable technique for tracking early autonomic changes in bleeding patients during progression to hemodynamic instability.

REFERENCES

1. Baillard C, Vivien B, Manisier P, et al. Brain death assessment using instant spectral analysis of heart rate variability. *Crit Care Med.* 2002;30:306–310.
2. Rapenne T, Moreau D, Lenfant F, et al. Could heart rate variability predict outcome in patients with severe head injury? A pilot study. *J Neurosurg Anesthesiol.* 2001;13:260–268.
3. Winchell RJ, Hoyt DB. Analysis of heart rate variability: a noninvasive predictor of death and poor outcome in patients with severe head injury. *J Trauma.* 1997;43:927–933.
4. Winchell RJ, Hoyt MD. Spectral analysis of heart rate variability in the ICU: a measure of autonomic function. *J Surg Res.* 1996;63:11–16.
5. Rothlisberger BW, Badra LJ, Hoag JB, et al. Spontaneous "baroreflex sequences" occur as deterministic functions of breathing phase. *Clin Physiol Funct Imag.* 2003;23:307–313.
6. Cooke WH, Ryan KL, Convertino VA. Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol.* 2004;96:1249–1261.
7. Brown TE, Beightol LA, Koh J, Eckberg DL. Important influence of respiration on human R-R interval power spectra is largely ignored. *J Appl Physiol.* 1993;75:2310–2317.
8. Cooke WH, Cox JF, Diedrich A, et al. Controlled breathing protocols probe human autonomic cardiovascular rhythms. *Am J Physiol.* 1998;274:H709–H718.
9. Akselrod S, Gordon D, Ubel FA, Shannon DC, Barger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science.* 1981;213:220–222.

10. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation.* 1996;93:1043–1065.

11. Grant RT, Reeve EB. Clinical observations of air raid casualties. *Br Med J.* 1941;2:293–297.

12. Shenkin HA, Cheney RH, Govons SR, Hardy JD, Fletcher AG Jr, Starr I. On the diagnosis of hemorrhage in man: a study of volunteers bled large amounts. *Am J Med Sci.* 1944;209:421–436.

13. Barcroft H, Edholm OG, McMichael J, Sharpey-Schafer EP. Posthaemorrhagic fainting study by cardiac output and forearm flow. *Lancet.* 1944;i:489–491.

14. McMichael J. Clinical aspects of shock. *JAMA.* 1944;29:275–281.

15. Cooke WH, Convertino VA. Association between vasovagal hypotension and low sympathetic neural activity during presyncope. *Clin Auton Res.* 2002;12:483–486.

16. Wallin BG, Nerhed C. Relationship between spontaneous variations of muscle sympathetic activity and succeeding changes of blood pressure in man. *J Auton Nerv Syst.* 1982;6:293–302.

17. Saul JP. Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *News Physiol Sci.* 1990;5:32–37.

18. Akselrod S. Components of heart rate variability: basic studies. In: Malik M, Camm AJ, ed. *Heart Rate Variability.* Armonk, NY: Futura Publishing Company; 1995:147–163.

19. Eiken O, Convertino VA, Doerr DF, Dudley G, Morariu G, Mekjavic IB. Interaction of the carotid baroreflex, the muscle chemoreflex, and the cardiopulmonary baroreflex in man during exercise. *Physiologist.* 1991;34(suppl):S118–S120.

20. Eiken O, Convertino VA, Doerr DF, Dudley GA, Morariu G, Mekjavic IB. Characteristics of the carotid baroreflex in man during normal and flow-restricted exercise. *Acta Physiol Scand.* 1991; 144:325–331.

21. Ludwig DA, Convertino VA. Predicting orthostatic intolerance: physics or physiology? *Aviat Space Environ Med.* 1994;65:404–411.

22. Convertino VA, Reister CA. Effects of G-suit protection on carotid–cardiac baroreflex function. *Aviat Space Environ Med.* 2000;71:31–36.

23. Thompson CA, Tatro DL, Ludwig DA, Convertino VA. Baroreflex responses to acute changes in blood volume in man. *Am J Physiol.* 1990;259:R792–R798.

24. Zöllei E, Paprika D, Makra P, Gingl Z, Vezendi K, Rudas L. Human autonomic responses to blood donation. *Auton Neurosci.* 2004; 110:114–120.

25. Faris IB, Jamieson GG, Ludbrook J. Effects of acute changes in blood volume on the carotid sinus baroreceptor reflex in conscious rabbits. *J Physiol.* 1983;337:563–573.

26. Ludbrook J, Faris IB, Jamieson GG. Blood volume and the carotid baroreceptor reflex in conscious rabbits. *Clin Sci.* 1981;61:173s–175s.

27. Randall WC, Kroeker T, Hotmire K, Burkholder T, Huprich S, Firth K. Baroreflex responses to the stress of severe hemorrhage in the rat. *Integr Physiol Behav Sci.* 1992;27:197–208.

28. Patwardhan AR, Vallurupalli S, Evans JM, Bruce EN, Knapp CF. Override of spontaneous respiratory pattern generator reduces cardiovascular parasympathetic influence. *J Appl Physiol.* 1995; 79:1048–1054.

29. Patwardhan AR, Evans JM, Bruce EN, Eckberg DL, Knapp CF. Voluntary control of breathing does not alter vagal modulation of heart rate. *J Appl Physiol.* 1995;78:2087–2094.

30. Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KUO, Eckberg DL. Human responses to upright tilt: a window on central autonomic integration. *J Physiol.* 1999;517:617–628.

31. Cooke WH, Dowlyn MM. Power spectral analysis imperfectly informs changes in sympathetic traffic during acute simulated microgravity. *Aviat Space Environ Med.* 2000;71:1232–1238.

32. Cooke WH, Pellegrini GL, Kovalenko OA. Dynamic cerebral autoregulation is preserved during acute head-down tilt. *J Appl Physiol.* 2003;95:1439–1445.